Figure S1: The Stp1 mutant is similar to WT for growth in TSB (A), human serum (B) and sialylation of the GBS capsular polysaccharide (C).
(A) Growth of GBS (WT, Δ*stp1*) in rich media such as TSB (Tryptic Soy Broth) was monitored by measuring the optical density at 600nm (O.D 600nm). Growth of the mutant was not significantly different from WT (*P*= 0.8).
(B) GBS (WT, Δ*stp1*) growth in normal human serum was measured for a period of 24 hrs as described previously (1). Overall growth of the Δ*stp1* was not significantly different from WT (*P=0.17*)
(C) Sialic acid concentrations in capsular polysaccharide isolated from WT GBS and the Δ*stp1* mutant was estimated as described (2). The amount of sialic acid obtained from CPS of WT and the Δ*stp1* mutant was not significantly different (*P*=0.7). NS= not significant.

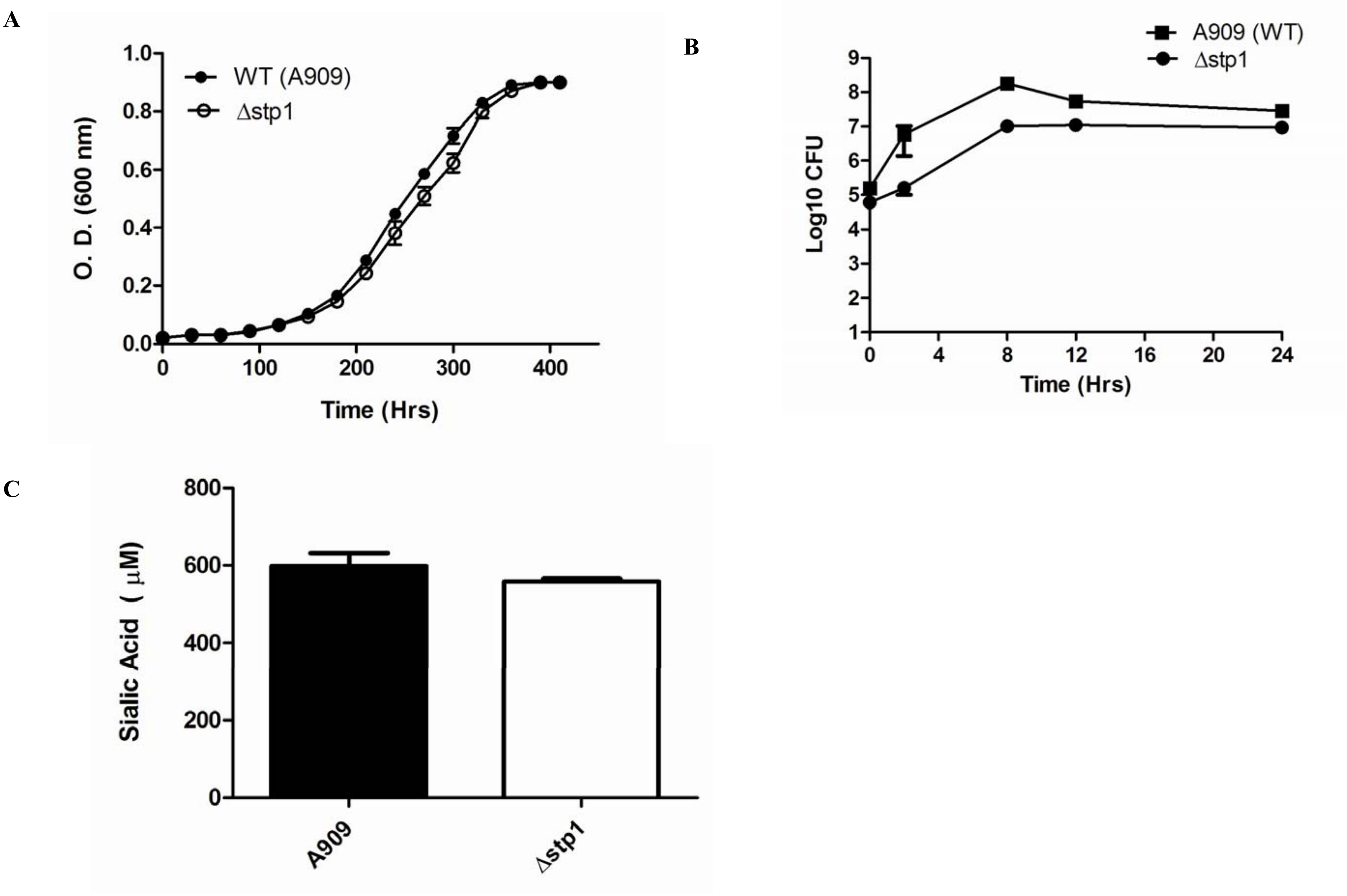


Figure S2: The Δ*stp1* mutant is proficient for adherence (A) and invasion (B) of human brain microvascular endothelial cells (hBMEC)
Adherence and Invasion of hBMEC by GBS A909 and the isogenic Δ*stp1* strain were performed at an MOI (multiplicity of infection) of 1 as described (3). Percent adherence was normalized to that of the WT as described (4). Note that the Δ*stp1* strain is similar to the WT for adherence and invasion of hBMEC. NS= not significant.

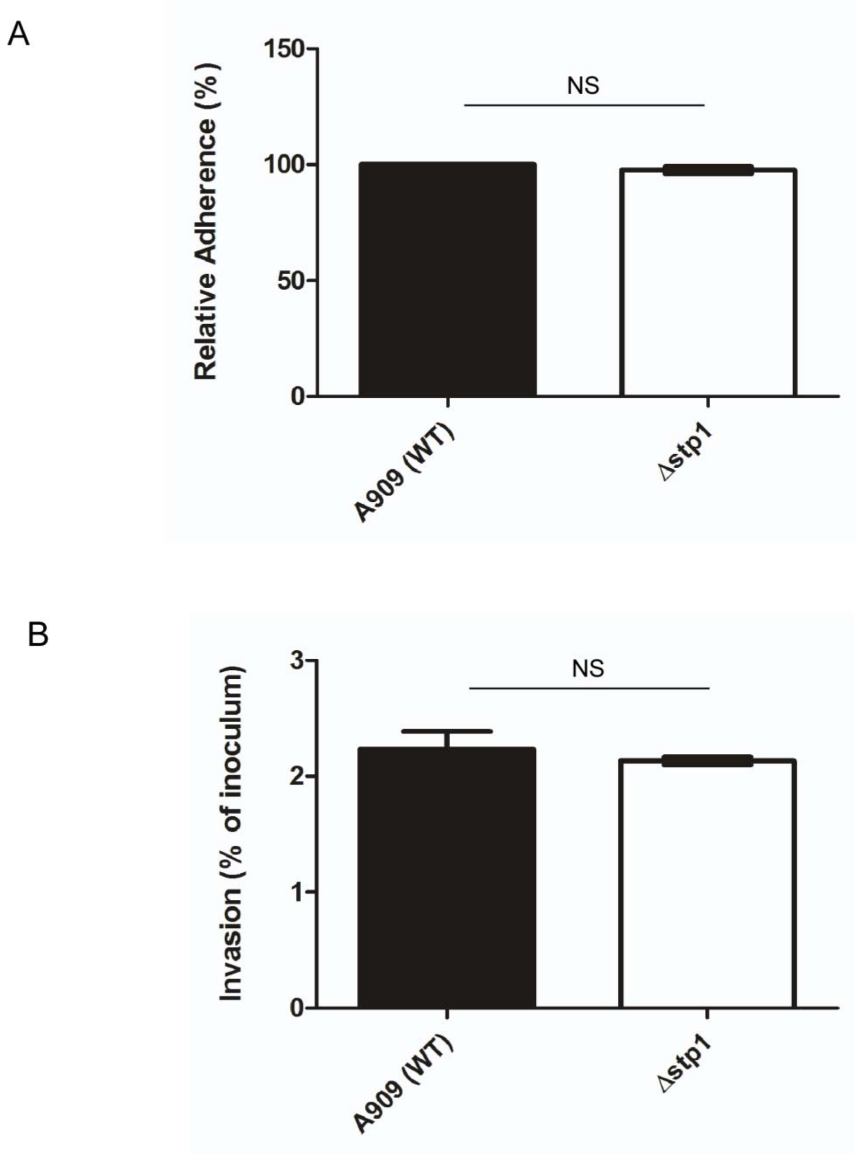
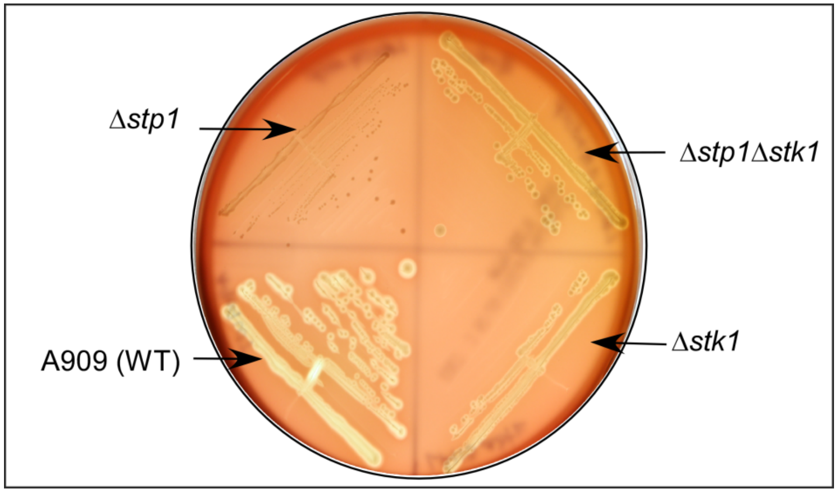


Figure S3

A. Hemolytic activity of GBS Δ*stp1* is lower than Δ*stk1* and Δ*stp1*Δ*stk1*

The zone of clearing observed around the colonies on the sheep blood agar plate represents β-H/C activity. Note that hemolytic activity of GBS deficient in expression of Stk1 (Δ*stk1*) and both Stp1 and Stk1 (Δ*stp1*Δ*stk1*) are similar and lower than WT A909 but greater than Δ*stp1*. The decrease in hemolytic activity in Δ*stk1* and Δ*stp1*Δ*stk1* correlated with decreased transcription of the *cylE* gene encoding hemolysin (5). The lack of hemolytic activity observed in Δ*stp1* is not due to decreased *cylE* transcription (see Table 3 of manuscript).



B. The increase in autolysis observed in GBSΔ*stp1* is not seen in GBS Δ*stp1*Δ*stk1* or Δ*stk1*.

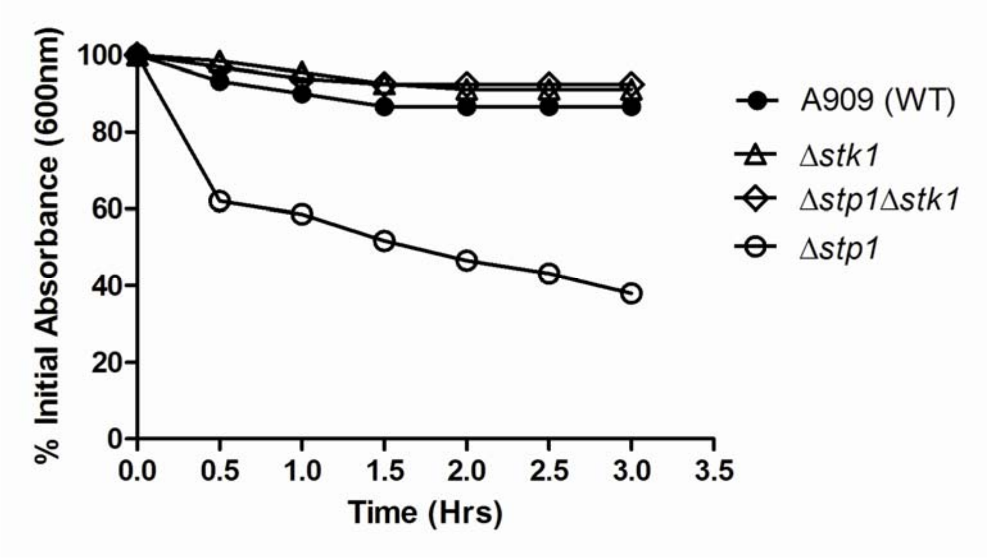


Table S1. Strains, plasmids and primers

Strains	Genotype\phenotype	Reference
Group B Streptococci or *S. agalactiae*		
A909	Wild-type (WT), Serotype Ia	(6)
LR154	Δ*stp1*::*Ωkm-2*\Km^{R}, A909	(7)
LR128	Δ*covR*::*sp*\Sp^{R}	(5)
LR155	Δ*covR* Δ*stp1*\Km^{R}, Sp^{R}	This study
LR113	Δ*stk1*::*Ωkm-2*\Km^{R}, A909	(8)
LR114	Δ*stp1*Δ*stk1*::*Ωkm-2*\Km^{R}, A909	(8)
LR171	Δ*SAK_0373/divIVA domain*::*Ωkm-2*\Km^{R}	This study
LR172	Δ*SAK_0581/ftsZ*::*Ωkm-2*\Km^{R}	This study
LR173	Δ*SAK_0586/divIVA*::*Ωkm-2*\Km^{R}	This study
Escherichia coli		
MC1061	F'*araD139* Δ*(ara-leu)7696* Δ*(lac)X74 galU galK hadR2* (r_k^- m_k^+) *mcrB1 rpsL* (Str^{+})	(9)
Plasmids		
pDC123	plasmid vector derived from pJS3, Cm^{R}	(10)
pStp1	pDC123 encoding Stp1	(8)

Primers	
PF3:5’	5-CGATCGTCTAGATGCATCAATAAAACGCCAAAACAAACGTTACTCTGT-3’
PR1+:5’	5’-CCAATTCACTGTTCCTTGCATGACGATGTCAGTTTCCTCCT -3’
NEWPF2+:5’	5’-AGTGAGGCGGTTTAATGATTCAGATTGGCAAATTATTTGCTG-3’
PR4:5’	5’-GCGTATGGTACCCACGGTGACTTTTCCAGCTTAGAGCAGTTGATAAATCAC -3’
PF1:5’	5’ -TGCAAGGAACAGTGAATTGGAGTTCGTCTTGTTATAATTA -3’
NEWPR2:5’	5’ -TAAACCGCCTCACTTTCAACAAACAATTCATCCAGTAAAA-3’
SAK_0373 5' XhoI	5'-CCATCTCGAGGCCATGTGCTCTATTTGGTG-3'
SAK_0373 5' + Kan 5'	5'-CTCCAATTCACTGTTCCTTGTCTATTCTCCAAACCAGTCA-3'
Kan 5' + SAK_0373 5'	5'-TGACTGGTTTGGAGAATAGACAAGGAACAGTGAATTGGAG-3'
Kan 3' + SAK_0373 3'	5'-GCATATTTGAGAAATCCTTATGCGGATGTACTTCAGAAAAG-3'
SAK_0373 3' + Kan 3'	5'-CTTTTCTGAAGTACTCCGCATAAGGATTTCTCAAATATGC-3'
SAK_0373 3' SpeI	5'-CCGTACTAGTCAATCAATGGCTTATCAGGG-3'
SAK_0581 5' XhoI	5'-CTCGCTCGAGGAGAAGTTATTTCATTGATTCCGC-3'
SAK_0581 5' + Kan 5'	5'-CTCCAATTCACTGTTCCTTGTTTTATTCCTCACTTATTGT-3'
Kan 5' + SAK_0581 5'	5'-ACAATAAGTGAGGAATAAAACAAGGAACAGTGAATTGGAG-3'
Kan 3' + SAK_0581 3'	5'-GTAAATTCATCATTTTAATTATGCGGATGTACTTCAGAAAAG-3'
SAK_0581 3' + Kan 3'	5'-CTTTTCTGAAGTACATCCGCATAATTAAAATGATGAATTTAC-3'
SAK_0581 3' XbaI	5'-CCTGTCTAGAGCATCCTCATACTTACGGGG-3'
SAK_0586 5' XhoI	5'-GGTCCTCGAGCTTATATCTTACTAGCCTATGCC-3'
SAK_0586 5' + Kan 5'	5'-CTCCAATTCACTGTTCCTTGGTTTTCTCCTTAGTTATGTATC-3'
Kan 5' + SAK_0586 5'	5'-GATACATAACTAAGGAGAAAACCAAGGAACAGTGAATTGGAG-3'
Kan 3' + SAK_0586 3'	5'-AATATTAAAACCTTGTTTTTCTTATGCGGATGTACTTCAGAAAAG-3'

SAK_0586 3' + Kan 3'	5'-CTTTTCTGAAGTACATCCGCATAAGAAAAACAAGGTTTTAATATT-3'
SAK_0586 3' SacII	5'-GGTTCCGCGGCTGGTCCTACTGTTAAACCGC-3'
qRT-PCR primers for select genes	
*cylE*L	5'-GGAAGTTACCCGATTGAGCA-3'
*cylE*R	5'-TGCCAGGAGGAGAATAGGAA-3'
*stk1*L	5'-GTATTGCGGTAGCGTTTGCT-3'
stk1R	5'-GCCTGCTCTGGCGATAAATA-3'
SAK_0652 qRT-F	5'-CTTTGATGGCTATTTTAGGAGC-3'
SAK_0652 qRT-R	5'-TGGCTCGTGGTAAGTCAATG-3'
SAK_0653 qRT-F	5'-GACGTTTTGGCGGTGATAGT-3'
SAK_0653 qRT-R	5'-CAGCGCTAGCGATTTTATCC-3'

Table S2 (A). Stk1 expression is similar to the WT in the Δ*stp1* mutant

Locus	**Gene Expression**	
	qRT-PCR	**Microarray***
stk1 (SAK_0389)	1.26 ± 0.11	1.08 ± 0.15

qRT-PCR was performed as described in *Experimental Procedures*. Gene expression is denoted as fold difference relative to the WT GBS strain A909 and standard deviation is indicated.* Indicates values from microarray analysis for *stk1* (see Table S5 for genes that showed > or < 2 fold change).

Table S2 (B). Stp1 regulated genes in GBS serotype Ia strain A909

(i): Upregulated genes			
Locus	**Protein description or name**	**Microarray Δ*stp1***	**qRT-PCR Δ*stp1***
Signal Transduction/Transcriptional regulators			
SAK_2031	transcriptional regulator Spx	6.14	5.1
SAK_0531	transcriptional regulator, AraC family	6.02	
SAK_0008	transcription-repair coupling factor	3.11	
SAK_1660	transcriptional regulator, putative	3.00	
SAK_0075	phosphosugar-binding transcriptional regulator, RpiR family	2.91	
SAK_1618	3H domain protein	2.62	
SAK_2067	response regulator	2.40	
SAK_0860	PspC domain protein	2.22	
SAK_2066	sensor histidine kinase, putative	2.12	
Transporters			
SAK_0565	BioY family protein	21.17	
SAK_1702	PTS system, sucrose-specific IIABC component	18.14	
SAK_1596	hydrophobic amino acid uptake (HAAT) family ABC transporter, permease protein LivM	17.22	
SAK_1597	hydrophobic amino acid uptake (HAAT) family ABC transporter, permease protein LivH	14.41	
SAK_1595	hydrophobic amino acid uptake (HAAT) family ABC transporter, ATP-binding protein LivG	11.15	
SAK_0472	BioY family protein	8.41	
SAK_0523	PTS system IIA domain protein	8.18	
SAK_0530	PTS system, galactitol-specific IIB component, putative	8.04	
SAK_0398	PTS system, IIA component, lactose/cellobiose family	6.59	
SAK_0529	PTS system, galactitol-specific IIC component	6.17	
SAK_1044	polar amino acid uptake (PAAT) family ABC transporter, amino acid-binding protein	5.42	

SAK_0256*	peptide/opine/nickel uptake (PepT) family ABC transporter, ATP-binding protein	3.40	3.5
SAK_0855	permease, putative	2.59	
SAK_1510	D-Serine/D-alanine/glycine:H+ symporter	2.54	
SAK_0354	PTS system, IIBC component	2.53	
SAK_0254*	peptide/opine/nickel uptake (PepT) family ABC transporter, permease protein	2.50	
SAK_1083	phosphate ABC transporter, ATP-binding protein PstB, putative	2.50	
SAK_1728	CvpA family protein	2.46	
SAK_0593	polar amino acid uptake (PAAT) family ABC transporter, ATP-binding protein	2.44	
SAK_1466	polar amino acid uptake (PAAT) family ABC transporter, amino acid-binding protein	2.43	
SAK_0207	oligopeptide ABC transporter, permease protein	2.39	
SAK_0173	ABC transporter, ATP-binding protein	2.36	
SAK_1085	phosphate ABC transporter, permease protein PtsA	2.25	
SAK_1082	phosphate transport system regulatory protein PhoU	2.24	
SAK_0940	probable proton-coupled thiamine transporter YuaJ	2.17	
SAK_1989	azaleucine resistance protein AzlD	2.16	
SAK_1086	phosphate ABC transporter, permease protein PstC	2.06	
SAK_1426*	ferrichrome ABC transporter, ferrichrome-binding protein	2.05	3.0
SAK_0259	PRD domain/PTS system IIA domain protein	2.05	
SAK_1646	nickel/cobalt uptake transporter (NiCoT) family ABC transporter, permease protein	2.04	
SAK_1593	CBS domain protein	7.99	17.94
SAK_1594	hydrophobic amino acid uptake (HAAT) family ABC transporter, ATP-binding protein LivF	7.98	
SAK_1895	PTS system, IIA component, putative	2.91	
SAK_0208	oligopeptide ABC transporter, permease protein OppC, putative	2.90	
SAK_1575	branched chain amino acid:H+ symporter	5.80	
SAK_0399	PTS system, IIB component, lactose/cellobiose family	7.77	
SAK_0255*	peptide/opine/nickel uptake (PepT) family ABC transporter, ATP-binding protein	2.77	
SAK_0439	purine transporter, AzgA family	2.77	

SAK_0664	calcium-transporting ATPase, P-type (transporting), HAD superfamily, subfamily IC	3.70	
SAK_0915	PTS system, beta-glucoside-specific IIABC component	3.69	
SAK_0400	PTS system, IIC component, lactose/cellobiose family	6.63	
SAK_0210	oligopeptide ABC transporter, ATP-binding protein	3.62	
SAK_1392	ABC transporter, permease protein, putative	2.62	
SAK_0534*	ABC transporter, permease protein	2.61	
SAK_0209	oligopeptide ABC transporter, ATP-binding protein	3.60	
Protein/peptide/amino acid and DNA synthesis/degradation			
SAK_0064	zoocin A, metallopeptidase	10.50	
SAK_0814	DNA-entry nuclease, putative	6.56	
SAK_1523	CAAX amino terminal protease family protein	6.16	
SAK_2120	B3/4 domain protein	4.58	
SAK_0007	peptidyl-tRNA hydrolase	3.59	
SAK_1386	16S rRNA-processing protein	2.91	
SAK_0284	replication initiation factor family protein	2.79	
SAK_0606	DNA-binding protein HU	2.78	
SAK_1815	50S ribosomal protein L34	2.56	
SAK_0054	CAAX amino terminal protease family protein	2.37	
SAK_0718	23S rRNA (uracil-5-)-methyltransferase RumA	2.17	
SAK_0570	endonuclease III	2.13	
SAK_1800	rRNA (guanine-N1-) methyltransferase (Mycinamicin-resistance protein)	2.03	
Cell envelope			
SAK_2106*	LysM domain protein	7.60	17.8
SAK_2105	transglycosylase-like domain protein	4.32	
SAK_0360	phospho-N-acetylmuramoyl-pentapeptide-transferase	3.56	
SAK_0250	LrgA family protein	3.07	
SAK_1206	polysaccharide deacetylase family protein	2.63	
SAK_0886	cell division protein, FtsW/RodA/SpoVE family	2.40	
SAK_1445	glycosyl transferase, group 1 family protein	2.38	
SAK_0358	cell division protein FtsL, putative	2.32	
SAK_0780	Cna B-type domain protein	2.11	
SAK_0782	Cna protein B-type domain	2.08	

Metabolic functions			
SAK_0566	biotin synthetase	14.67	
SAK_1171	xanthine phosphoribosyltransferase	12.91	
SAK_1701	fructokinase	11.39	
SAK_0568	acetyl-CoA acetyltransferase family protein	9.37	
SAK_1170	xanthine permease	6.95	
SAK_0527	rhamnulose-1-phosphate aldolase	3.75	
SAK_1110	carbon starvation protein CstA	3.74	
SAK_0539	aldose 1-epimerase, interruption-N	3.66	
SAK_0538	UDP-glucose 4-epimerase	3.48	
SAK_0401	formate acetyltransferase 2	3.26	
SAK_0402	fructose-6-phosphate aldolase	3.23	
SAK_0537	galactose-1-phosphate uridylyltransferase	2.95	
SAK_1672	isochorismatase family protein	2.88	
SAK_0198	glycosyl transferase family protein	2.83	
SAK_1673	HD domain protein	2.81	
SAK_0187*	isoprenylcysteine carboxyl methyltransferase (ICMT) family protein	2.60	
SAK_1674	nicotinic acid mononucleotide adenyltransferase	2.56	
SAK_1662	dihydroxyacetone kinase DAK1 domain protein	2.50	
SAK_1663	dihydroxyacetone kinase DAK2 domain protein	2.44	
SAK_1703	sucrose-6-phosphate hydrolase	2.28	
SAK_0345	glycerol kinase	2.20	
SAK_1057	glyoxylase family protein	2.11	
Mobile genetic elements			
SAK_0695	ISSag6, transposase orfA	2.04	
Autolysis			
SAK_0652	prophage LambdaSa03, holin, phi LC3 family	50.17	718
SAK_0653	prophage LambdaSa03, peptidoglycan endolysin	31.00	370
Unknown function			
SAK_0651	hypothetical protein	32.67	
SAK_1522	hypothetical protein	8.47	
SAK_0440*	conserved hypothetical protein TIGR00150	8.07	10.2

SAK_0567	hypothetical protein	7.59	
SAK_0569	hypothetical protein	7.39	
SAK_0856	hypothetical protein	3.76	
SAK_0372	hypothetical protein	3.43	
SAK_1599	hypothetical protein	3.19	
SAK_0443	hypothetical protein	3.03	
SAK_0854	hypothetical protein	2.85	
SAK_0605	hypothetical protein	2.85	
SAK_2089	prophage Sa05, BRO domain protein	2.84	
SAK_1664	hypothetical protein	2.82	
SAK_0283	hypothetical protein	2.79	
SAK_2038	hypothetical protein	2.71	
SAK_1012	hypothetical protein	2.70	
SAK_0344	hypothetical protein	2.55	
SAK_0762	prophage LambdaSa04, LysM domain protein	2.50	
SAK_2073	prophage Sa05, membrane protein, putative	2.49	
SAK_2130	hypothetical protein	2.47	
SAK_1016	integral membrane protein TIGR01906	2.44	
SAK_1533	hypothetical protein	2.37	
SAK_1675	conserved hypothetical protein TIGR00253	2.35	
SAK_1671	iojap-related protein	2.34	
SAK_2072	hypothetical protein	2.30	
SAK_1729	hypothetical protein	2.25	
SAK_1058	hypothetical protein	2.18	
SAK_0990	hypothetical protein	2.15	
SAK_1640	hypothetical protein	2.10	
SAK_0232	hypothetical protein	2.10	

(ii): Downregulated genes

Locus	Protein description or name	Microarray Δ*stp1*	qRT-PCR Δ*stp1*
Pathogenesis			
SAK_0517	C protein alpha-antigen	0.41	
SAK_0955*	fibrinogen-binding protein, FbsB	0.16	0.1
SAK_1142*	fibrinogen-binding protein, FbsA	0.13	
Signal Transduction/Transcriptional regulators			
SAK_0279	DNA-binding protein	0.49	
SAK_1220	transcriptional regulator, GntR family/TrkA domain protein	0.46	
SAK_2012	transcriptional regulator, GntR family	0.43	
SAK_0381	DNA-binding response regulator, LuxR family	0.35	
SAK_1397	pyrimidine regulatory protein PyrR	0.35	
SAK_1828	sugar-binding transcriptional regulator, LacI family	0.30	
SAK_0928	competence protein CoiA, putative	0.27	
SAK_0380*	sensor histidine kinase	0.25	
SAK_1363	transcriptional regulator, MarR family	0.17	
SAK_0388	serine/threonine protein phosphatase Stp1	0.0027	
Secretion systems/Transporters			
SAK_1336	ATP-dependent Clp protease, ATP-binding subunit ClpL	0.43	
SAK_1361	efflux ABC transporter, permease/ATP-binding protein	0.40	
SAK_1396	carbamoyl-phosphate synthase small subunit	0.34	
SAK_1395	carbamoyl-phosphate synthase, large subunit, putative	0.30	
SAK_1554	metal ABC transporter, permease protein	0.29	
SAK_1817	glycine betaine/proline ABC transporter, permease/substrate-binding protein	0.29	
SAK_1818	glycine betaine/proline ABC transporter, ATP-binding protein	0.28	
SAK_1362	efflux ABC transporter, permease/ATP-binding	0.25	

	protein		
SAK_1716	drug:H+ antiporter-2 (DHA2) family protein	0.24	
SAK_1555	metal ABC transporter, ATP-binding protein	0.23	
SAK_0378	ABC transporter, ATP-binding protein	0.20	
SAK_1556	metal ABC transporter, metal-binding lipoprotein	0.15	
SAK_1656	amino acid ABC transporter, amino acid-binding protein, putative	0.14	
SAK_1996	major facilitator family protein	0.12	
SAK_1858	peptidase, U61 (muramoyl-tetrapeptide carboxypeptidase) family	0.50	
SAK_1222	CsbD family protein	0.41	
SAK_1223	alkaline shock protein 23, putative	0.40	
SAK_0774	chaperone protein HslO, putative	0.39	
SAK_1221	alkaline shock protein 23, putative	0.39	
SAK_0150	tRNA pseudouridine synthase A	0.37	
SAK_2014	co-chaperonin GroES	0.37	
SAK_0804	peptidase, S8 (subtilisin) family	0.15	
Cell envelope			
SAK_1234	LrgB family protein	0.43	
SAK_1737	beta-lactamase, putative	0.42	
SAK_0948	polysaccharide deacetylase family protein	0.41	
SAK_1233	LrgA family protein	0.37	
SAK_0066	N-acetylmannosamine-6-phosphate 2-epimerase	0.37	
SAK_1655	lipoprotein, NLPA family	0.36	
SAK_1226	transglycosylase associated protein	0.34	
SAK_0338	N-acetylglucosamine-6-phosphate deacetylase	0.31	
SAK_1227	transglycosylase associated protein	0.22	
SAK_1997	patatin-like phospholipase family protein	0.17	
SAK_1570*	glycosyl transferase, group 2 family protein	0.15	
Metabolic functions			
SAK_0058	phosphoribosylformylglycinamidine synthase	0.38	
SAK_0059	amidophosphoribosyltransferase	0.41	
SAK_0060	phosphoribosylaminoimidazole synthetase	0.47	
SAK_0151	phosphomethylpyrimidine kinase	0.44	
SAK_0328	acetyltransferase, GNAT family	0.43	

SAK_0496	acetyltransferase, GNAT family	0.31	
SAK_0657	dihydroorotate dehydrogenase	0.15	
SAK_0924	glucosamine-6-phosphate isomerase	0.25	
SAK_0925	glutathione S-transferase domain protein	0.15	
SAK_1132	carbamoyl-phosphate synthase large subunit	0.12	
SAK_1133	carbamoyl-phosphate synthase small subunit	0.07	
SAK_1134	aspartate carbamoyltransferase catalytic subunit	0.10	
SAK_1135	dihydroorotase	0.15	
SAK_1136	orotate phosphoribosyltransferase	0.11	
SAK_1137	orotidine 5'-phosphate decarboxylase	0.18	
SAK_1141*	aspartate-semialdehyde dehydrogenase	0.25	
SAK_1230	uracil permease	0.25	
SAK_1505	RNA pseudouridine synthase family protein	0.37	
SAK_1584	D-3-phosphoglycerate dehydrogenase, putative	0.20	
SAK_1585	acetyltransferase, GNAT family	0.18	
SAK_1651	alcohol dehydrogenase, zinc-containing	0.43	
SAK_1657	glutamine amidotransferase class I domain protein	0.17	
SAK_2010	concentrative nucleoside transporter (CNT) family protein	0.44	
SAK_2011	uridine phosphorylase	0.24	
SAK_2022	acetyltransferase, GNAT family	0.30	
SAK_2023	oxidoreductase, Gfo/Idh/MocA family	0.40	
SAK_2025	anaerobic ribonucleoside triphosphate reductase	0.41	
Unknown function			
SAK_1904	hypothetical protein	0.50	
SAK_1738	hypothetical protein	0.48	
SAK_1126	hypothetical protein	0.47	
SAK_1808*	hypothetical protein	0.46	0.3
SAK_0839*	hypothetical protein	0.46	
SAK_0152	hypothetical protein	0.46	
SAK_2094	prophage Sa05, site-specific recombinase, phage integrase family	0.45	
SAK_1807*	hypothetical protein	0.45	
SAK_0759	prophage LambdaSa04, minor structural protein, putative	0.44	
SAK_0678	hypothetical protein	0.44	

SAK_1216	hypothetical protein	0.41	
SAK_1224	hypothetical protein	0.41	
SAK_0729	hypothetical protein	0.38	
SAK_0495	hypothetical protein	0.37	
SAK_1129	hypothetical protein	0.37	
SAK_0589	hydrolase, NUDIX family	0.36	
SAK_1128	hypothetical protein	0.34	
SAK_1982	rhodanese-like domain protein	0.34	
SAK_0756	prophage LambdaSa04, tail tape measure protein, TP901 family	0.33	
SAK_0748	prophage LambdaSa04, major capsid protein, HK97 family	0.32	
SAK_0758	prophage LambdaSa04, minor structural protein	0.32	
SAK_0757	prophage LambdaSa04, tail protein, putative	0.30	
SAK_0755	hypothetical protein	0.30	
SAK_0656	hypothetical protein	0.29	
SAK_0730	prophage LambdaSa04, DNA primase, P4 family	0.29	
SAK_2093	hypothetical protein	0.27	
SAK_2024	hypothetical protein	0.27	
SAK_2092	hypothetical protein	0.25	
SAK_0742	prophage LambdaSa04, terminase, large subunit	0.24	
SAK_2091	prophage Sa05, DNA-binding protein	0.23	
SAK_0752	hypothetical protein	0.21	
SAK_0746	prophage LambdaSa04, portal protein, HK97 family	0.21	
SAK_0628*	hypothetical protein	0.20	
SAK_0740	hypothetical protein	0.19	
SAK_0739	prophage LambdaSa04, methyltransferase, C-5 cytosine-specific family	0.19	
SAK_0956*	hypothetical protein	0.18	
SAK_0379*	hypothetical protein	0.18	
SAK_0750	prophage LambdaSa04, head-tail adaptor, putative	0.18	
SAK_0738	prophage LambdaSa04, DNA methylase	0.18	
SAK_0741	prophage LambdaSa04, terminase, small subunit, P27 family	0.17	
SAK_1571*	hypothetical protein	0.16	0.1
SAK_0627*	hypothetical protein	0.16	
SAK_1573*	hypothetical protein	0.15	

SAK_1572*	hypothetical protein	0.15	
SAK_0728	hypothetical protein	0.14	
SAK_0802	hypothetical protein	0.14	
SAK_0803	hypothetical protein	0.13	
SAK_0608*	hypothetical protein	0.09	
SAK_1130	hypothetical protein	0.08	
SAK_0612*	hypothetical protein	0.08	
SAK_1131	hypothetical protein	0.05	
SAK_0631*	hypothetical protein	0.04	
SAK_0641*	hypothetical protein	0.04	
SAK_0649*	hypothetical protein	0.04	
SAK_0630*	prophage LambdaSa03, HNH endonuclease family protein	0.04	
SAK_0629*	hypothetical protein	0.04	
SAK_0626*	hypothetical protein	0.04	
SAK_0643*	hypothetical protein	0.03	
SAK_0625*	hypothetical protein	0.03	
SAK_0634*	hypothetical protein	0.03	
SAK_0650*	hypothetical protein	0.03	
SAK_0642*	prophage LambdaSa03, structural protein, putative	0.02	
SAK_0611*	prophage LambdaSa03, transcriptional regulator, Cro/CI family	0.02	
SAK_0639*	hypothetical protein	0.02	
SAK_0638*	hypothetical protein	0.02	
SAK_0644*	hypothetical protein	0.02	
SAK_0645*	prophage LambdaSa03, pblA protein, internal deletion	0.02	
SAK_0609*	hypothetical protein	0.02	
SAK_0615*	hypothetical protein	0.02	
SAK_0636*	hypothetical protein	0.02	
SAK_0621*	hypothetical protein	0.02	
SAK_0646*	prophage LambdaSa03, tail component, putative	0.01	
SAK_0622*	hypothetical protein	0.01	
SAK_0640*	hypothetical protein	0.01	
SAK_0633*	hypothetical protein	0.01	
SAK_0648*	prophage LambdaSa03, minor structural protein, putative	0.01	

SAK_0614*	hypothetical protein	0.01	
SAK_0620*	hypothetical protein	0.01	
SAK_0619*	conserved hypothetical protein/bacteriophage resistance protein	0.01	
SAK_0610*	prophage LambdaSa03, transcriptional regulator, Cro/CI family	0.01	
SAK_0617*	prophage LambdaSa03, helicase, putative	0.01	
SAK_0618*	hypothetical protein	0.01	
SAK_0647*	prophage LambdaSa03, minor structural protein, putative	0.01	
SAK_0637*	prophage LambdaSa03, structural protein, putative	0.01	
SAK_0635*	prophage LambdaSa03, terminase, large subunit, putative	0.01	
SAK_0607*	prophage LambdaSa03, site-specific recombinase, phage integrase family	0.01	
SAK_0624*	hypothetical protein	0.01	
SAK_0623*	hypothetical protein	0.01	
SAK_0616*	conserved hypothetical protein TIGR01618	0.01	
SAK_0632*	hypothetical protein	0.004	

*** indicates CovR regulated genes**

Table S3. S/T phosphopeptides that showed neutral loss of phosphoric acid during CID from GBS Δ*stp1* and WT A909

S/T peptides identified in Δ*stp1*	**Protein Accessions**	**Modifications**	**Protein Description**	**Observed m/z**	**Mono m/z**	**MH+**	**Z**	**Δ score**
IAATDSVINtLSGQQAAAQK	SAK_0050	T10(Phospho)	PcsB protein (pcsB)	984.9	1034.01	2067.01	2	0.06
FQAAAGQLEKtAR	SAK_0099	T11(Phospho)	ribosomal protein L29 (rpmC)	686.93	735.86	1470.7	2	0.06
VNVNtEcQLAFcQATR	SAK_0178	T5(Phospho)	fructose-1,6-bisphosphate aldolase, class II (fba) [4.1.2.13]	947.33	995.93	1990.85	2	0.04
IYYtHSMYPGGLK	SAK_0276	T4(Phospho)	ribosomal protein L13 (rplM)	756.41	805.35	1609.71	2	0.05
LDtEMIGLVK	SAK_0361	T3(Phospho)	ATP-dependent RNA helicase, DEAD/DEAH box family	550.84	599.79	1198.5	2	0.03
NSGtAMYNQKPIAQSATNFDILK	SAK_0373	T4(Phospho)	DivIVA domain protein	832.89	864.74	2592.22	3	0.01
AGItEEDSILDK	SAK_0375	T4(Phospho)	conserved hypothetical protein	637.11	685.80	1370.61	2	0.07
SSDFANLDtASLDDFIK	SAK_0375	T9(Phospho)	conserved hypothetical protein	921.25	969.92	1938.8	2	0.06
LVFNDTEStKTLPK	SAK_0389	T9(Phospho)	serine/threonine protein kinase Stk1 (stk1)	788.23	838.91	1672.8	2	0.06
VTSTVSSLttEQLLR	SAK_0389	T9(Phospho)	serine/threonine protein kinase Stk1 (stk1)	848.85	897.92	1794.83	2	0.09
sLGNGIDPMDVIEK	SAK_0547	S1(Phospho)	valyl-tRNA synthetase (valS)	735.80	784.35	1567.71	2	0.09
DNISRPtEGELDSK	SAK_0581	T7(Phospho)	cell division protein FtsZ (ftsZ)	772.2	820.86	1640.7	2	0.08
KDKTNQVSGFtTSAPTNQAPSER	SAK_0581	T11(Phospho)	cell division protein FtsZ (ftsZ)	816.34	848.73	2544	3	0.01
TGQEtSFDFDMK	SAK_0583	T5(Phospho)	conserved hypothetical protein	694.64	743.28	1485.56	2	0.08
ESLSQSVILAQEtAER	SAK_0586	T13(Phospho)	cell division protein DivIVA, putative	884.53	920.94	1840.87	2	0.05
QLEESGLLDtNNFQMEEPINLGETQTFK	SAK_0586	T9(Phospho)	cell division protein DivIVA	1070.73	1102.50	3305.50	3	0.08
LEtGDVALEDAIAEFQK	SAK_0598	T3(Phospho)	exodeoxyribonuclease VII, small subunit (xseB)	916.81	964.94	1928.88	2	0.03
LGKtEDDIIVNK	SAK_0651	T4(Phospho)	conserved hypothetical protein	664.19	712.85	1424.70	2	0.05

AEEHtIALGQItEQIPAIVAK	SAK_0709	T5(Phospho)	septation ring formation regulator EzrA (ezrA)	766.16	798.05	2392.16	3	0.05
VTHALDLYEtLQK	SAK_0709	T10(Phospho)	septation ring formation regulator EzrA (ezrA)	797.34	845.88	1690.75	2	0.08
TsEVPVAEDDSFLELER	SAK_0853	S2(Phospho)	signal recognition particle-docking protein FtsY (ftsY)	959.89	1008.45	2015.89	2	0.00
LTHLIsQNEVNDD	SAK_0862	S6(Phospho)	HPr kinase/phosphorylase (hprK)	740.35	789.34	1577.68	2	0.05
APEtKVEDIVIDYK	SAK_0865	T4(Phospho)	conserved hypothetical protein	801.79	850.41	1699.82	2	0.04
DKASEYsNLAVDTFK	SAK_0865	S7(Phospho)	conserved hypothetical protein	835.82	884.40	1767.79	2	0.08
FESGELTtEDIVSAVK	SAK_0865	T8(Phospho)	conserved hypothetical protein	845.03	902.91	1804.83	2	0.02
GKFEsGELTTEDIVSAVK	SAK_0865	S5(Phospho)	conserved hypothetical protein	946.89	995.47	1989.94	2	0.04
GLDtGFYDFDPSTVK	SAK_0867	T4(Phospho)	peptidase, U32 (collagenase) family	822.79	871.37	1741.74	2	0.06
DTDKPLLLPVEDVFSItGR	SAK_0887	T17(Phospho)	translation elongation factor Tu (tuf)	700.79	732.37	2195.10	3	0.05
REEELSNAKtEANQIIDNAK	SAK_0982	T10(Phospho)	ATP synthase F0, B subunit (atpF)	751.75	785.04	2353.10	3	0.09
ELEAFtQFGSDLDAATQAK	SAK_0984	T6(Phospho)	ATP synthase F1, alpha subunit (atpA)	1013.43	1061.47	2121.94	2	0.02
IGHtAYQVTQNSATEHAFTGK	SAK_1026	T4(Phospho)	methionine-R-sulfoxide reductase (msrB)	748.72	781.02	2341.06	3	0.04
EQPtQFGQGMSLQQALQAR	SAK_1228	T4(Phospho)	ATP-dependent DNA helicase PcrA (pcrA)	1050.93	1099.50	2198.00	2	0.05
VSGQTILDQEtK	SAK_1559	T11(Phospho)	conserved hypothetical protein	650.90	699.83	1398.85	2	0.03
SSEFRtTENVPDIDLK	SAK_1628	T6(Phospho)	conserved hypothetical protein TIGR00247	917.30	965.96	1930.88	2	0.01
tAQLMADYEAQR	SAK_1628	T1(Phospho)	conserved hypothetical protein TIGR00247	690.16	738.81	1476.62	2	0.02
FSDQEtKEFASSLSK	SAK_1681	T6(Phospho)	glutamyl-tRNA(Gln) amidotransferase, C subunit (gatC)	843.76	892.39	1783.78	2	0.01
MTtENLGEIVISPR	SAK_1706	T3(Phospho)	conserved hypothetical protein	771.37	820.39	1639.78	2	0.03
NTEItRLYEQLK	SAK_1774	T5(Phospho)	conserved hypothetical protein	745.80	794.39	1587.78	2	0.07

TILEEEPIDEEAsRR	SAK_1774	S13(Phospho)	conserved hypothetical protein	885.36	933.92	1866.85	2	0.05
sQMEATTSDFDREK	SAK_2013	S1(Phospho)	chaperonin GroEL (groEL)	814.68	862.84	1724.68	2	0.01
LTAPSVQFDEtTGDYSR	SAK_2048	T11(Phospho)	ribosomal protein L32 (rpmF)	935.98	983.92	1966.84	2	0.05
IYYtHSMYPGGLK	SAK_0276	T4(Phospho)	ribosomal protein L13 (rplM)	756.41	805.35	1609.71	2	0.05
S/T peptides identified in WT A909	**Protein Accessions**	**Modifications**	**Protein Description**	**Observed m/z**	**Mono m/z**	**MH+**	**Z**	**Δ score**
sIWESQKEPIQEAITSFK	SAK_0186	S1(Phospho)	IgA-binding beta antigen (bag)	702.42	734.35	2201.05	3	0.08
sLQDFIPLNEGK	SAK_0270	S1(Phospho)	cysteinyl-tRNA synthetase (cysS)	672.34	720.84	1440.68	2	0.01
VTSTVSSLTtEQLLR	SAK_0389	T10(Phospho)	serine/threonine protein kinase Stk1 (stk1)	808.98	857.93	1714.86	2	0.06
sQFLQGSWNYER	SAK_0433	S1(Phospho)	PTS system, IID component, mannose/fructose/sorbose family	749.24	797.83	1594.66	2	0.09
sLQLLAQNYLHDR	SAK_0483	S1(Phospho)	R3H domain protein	777.32	825.90	1650.80	2	0.01
sLGNGIDPMDVIEK	SAK_0547	S1(Phospho)	valyl-tRNA synthetase (valS)	735.37	784.36	1567.71	2	0.04
VLDEDDALPVVDDTESFDAtR	SAK_0586	T20(Phospho)	cell division protein DivIVA, putative	1271.01	1303.23	3907.68	3	0.07
LTHLIsQNEVNDD	SAK_0862	S6(Phospho)	HPr kinase/phosphorylase (hprK)	740.35	789.34	1577.68	2	0.05
sFEGLYDLHNK	SAK_0897	S1(Phospho)	peptide chain release factor 3 (prfC)	653.15	701.80	1402.60	2	0.06
sFDFITK	SAK_1544	S1(Phospho)	ribosomal protein L11 (rplK)	420.49	469.20	937.40	2	0.05
VSGQTILDQEtK	SAK_1559	T11(Phospho)	conserved hypothetical protein	650.90	699.83	1398.65	2	0.09
FSDQEtKEFASSLSK	SAK_1681	T6(Phospho)	glutamyl-tRNA(Gln) amidotransferase, C subunit (gatC)	843.76	892.39	1783.78	2	0.09
sVGGFVLAGASHDATK	SAK_2014	S1(Phospho)	chaperonin GroES (groES)	750.25	798.87	1596.74	2	0.02

Phosphopeptide enrichment was performed as described previously ((11), also see *Experimental Procedures*). Phosphopeptides below showed neutral loss of phosphoric acid in the mass spectrometric (MS) analysis. The phosphorylated threonine or serine residue is indicated in small letter ‘t’ or ‘s’. SAK numbers correspond to the ORF of the gene in the GBS A909 genome (12). The mass difference between observed m/z and mono m/z indicates the loss of phosphoric acid from double (49Da) or triple (~32.6Da) charged peptides.

Table S4. Virulence of GBS lacking FtsZ, DivIVA, and DivIVA domain

Moribund 50 (MD_{50}) estimates and confidence intervals were calculated as described in the *Experimental Procedures*. *P* value compares the value of the mutant strain to the WT A909 (*P* value < 0.05 are considered significant).

Strain	**MD_{50} (CFU)**	**95% confidence interval**	***P* value**
A909 (WT)	1.5×10^4	$2.1 \times 10^3 - 1.1 \times 10^5$	N/A
Δ*ftsZ*	2.4×10^4	$4.7 \times 10^3 - 1.2 \times 10^5$	0.7
Δ*divIVA*	1.9×10^5	$2.8 \times 10^4 - 1.2 \times 10^6$	0.09
Δ*divIVA domain*	2.6×10^5	$3.9 \times 10^4 - 1.5 \times 10^6$	0.06

References

1. Lamy, M. C., Zouine, M., Fert, J., Vergassola, M., Couve, E., Pellegrini, E., Glaser, P., Kunst, F., Msadek, T., Trieu-Cuot, P., and Poyart, C. (2004) *Mol. Microbiol.* **54**, 1250-1268
2. Chaffin, D. O., Mentele, L. M., and Rubens, C. E. (2005) *J. Bacteriol.* **187**, 4615-4626
3. Doran, K. S., Liu, G. Y., and Nizet, V. (2003) *J. Clin. Invest.* **112**, 736-744
4. Doran, K. S., Engelson, E. J., Khosravi, A., Maisey, H. C., Fedtke, I., Equils, O., Michelsen, K. S., Arditi, M., Peschel, A., and Nizet, V. (2005) *J. Clin. Invest.* **115**, 2499-2507
5. Rajagopal, L., Vo, A., Silvestroni, A., and Rubens, C. E. (2006) *Mol. Microbiol.* **62**, 941-957
6. Madoff, L. C., Michel, J. L., Gong, E. W., Kling, D. E., and Kasper, D. L. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 4131-4136
7. Lin, W. J., Walthers, D., Connelly, J. E., Burnside, K., Jewell, K. A., Kenney, L. J., and Rajagopal, L. (2009) *Mol. Microbiol.* **71**, 1477-1495
8. Rajagopal, L., Clancy, A., and Rubens, C. E. (2003) *J. Biol. Chem.* **278**, 14429-14441
9. Wertman, K. F., Wyman, A. R., and Botstein, D. (1986) *Gene* **49**, 253-262
10. Chaffin, D. O., and Rubens, C. E. (1998) *Gene* **219**, 91-99
11. Burnside, K., Lembo, A., de Los Reyes, M., Iliuk, A., Binhtran, N. T., Connelly, J. E., Lin, W. J., Schmidt, B. Z., Richardson, A. R., Fang, F. C., Tao, W. A., and Rajagopal, L. (2010) *PLoS ONE* **5**, e11071
12. Tettelin, H., Masignani, V., Cieslewicz, M. J., Donati, C., Medini, D., Ward, N. L., Angiuoli, S. V., Crabtree, J., Jones, A. L., Durkin, A. S., Deboy, R. T., Davidsen, T. M., Mora, M., Scarselli, M., Margarit y Ros, I., Peterson, J. D., Hauser, C. R., Sundaram, J. P., Nelson, W. C., Madupu, R., Brinkac, L. M., Dodson, R. J., Rosovitz, M. J., Sullivan, S. A., Daugherty, S. C., Haft, D. H., Selengut, J., Gwinn, M. L., Zhou, L., Zafar, N., Khouri, H., Radune, D., Dimitrov, G., Watkins, K., O'Connor, K. J., Smith, S., Utterback, T. R., White, O., Rubens, C. E., Grandi, G., Madoff, L. C., Kasper, D. L., Telford, J. L., Wessels, M. R., Rappuoli, R., and Fraser, C. M. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102**, 13950-13955